

Materials List for:

Fabrication of a Multiplexed Artificial Cellular MicroEnvironment Array

Yasumasa Mashimo^{1,2}, Momoko Yoshioka¹, Yumie Tokunaga¹, Christopher Fockenber¹, Shiho Terada¹, Yoshie Koyama¹, Teiko Shibata-Seki², Koki Yoshimoto¹, Risako Sakai¹, Hayase Hakariya¹, Li Liu¹, Toshihiro Akaiki³, Eiry Kobatake², Siew-Eng How⁴, Motonari Uesugi^{1,5}, Yong Chen^{1,6}, Ken-ichiro Kamei¹

¹Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University

²Department of Life Science and Technology, School of Life Science and Technology, Tokyo Institute of Technology

³Biomaterials Center for Regenerative Medical Engineering, Foundation for Advancement of International Science

⁴Faculty of Science and Natural Resources, Universiti Malaysia Sabah

⁵Institute for Chemical Research, Kyoto University

⁶Ecole Normale Supérieure

Correspondence to: Ken-ichiro Kamei at kkamei@icems.kyoto-u.ac.jp

URL: <https://www.jove.com/video/57377>

DOI: [doi:10.3791/57377](https://doi.org/10.3791/57377)

Materials

Name	Company	Catalog Number	Comments
Polystyrene (PS)	Sigma	#182435	Average Mw: 290,000, average Mn: 130,000
Polymethylglutarimide (PMGI)	MicroChem	G113113	
Gelatin (GT)	Sigma	G2625	From porcine skin, type A
Sylgard 184 silicone elastomer kit	Doe Corning Toray	#1064291	PDMS curing agent and silicone elastomer base are components of this kit.
OpenSCAD			This is a free 3D computer graphics software (http://www.openscad.org/) used for designing the mold of the microfluidic device.
AutoCAD 2014	Autodesk		This is a 3D computer graphics software (https://www.autodesk.com/products/autocad/overview) used for design of the mask used on nanofiber-array preparation.
3D printer, AGILISTA-3000	Keyence		
UV-curable resin, AR-M2	Keyence		This is used for 3D printing by Agilista.
Acetic acid	Sigma	#338826	≥99.99%
Ethyl acetate	Sigma	#270989	Anhydrous, 99.8%
Tetrahydrofuran (THF)	Sigma	#401757	
MSP-30T	Vacuum Device		Magnetron sputtering machine
Nunc OmniTray	Thermo Fisher Scientific	#242811	This is a polystyrene baseplate on which the nanofiber array is created. This plate size is typically 127.7 x 85.5 mm.
Gun-type corona discharge machine	Shinko Electric & Instrumentation	CFG-500	This handy device is used to generate corona for activation of the bottom surface of the PDMS layer at step 1.5 "Assembly of the MACME arrays" in the protocol.
5 mL syringe	Terumo	SS-05SZ	

Stainless-steel blunt needle (23-gauge)	Nipro	#2166	Outside diameter and length are 0.6 and 32 mm, respectively.
High-voltage power supply	TechDempaz		
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride	Dojindo	W001	
N-Hydroxysuccinimide	Sigma	#56480	
Matrigel hESC-Qualified Matrix	Corning	#354277	This protein is referred as basement membrane gel matrix in the protocol.
CellAdhere Vitronectin, Human, Solution	STEMCELL Technologies	#07004	
TeSR-E8	STEMCELL Technologies	#05940	Feeder-free, xeno-free culture medium for maintenance of human ES and iPS cells
Y-27632	Wako Pure Chemical Industries	#253-00513	
TrypLE Express Enzyme (1X), phenol red	Thermo Fisher Scientific	#12605028	This is a recombinant trypsin-like protease for dissociation of adherent mammalian cells.
Click-iT EdU Imaging Kit with Alexa Fluor 647 Azides	Thermo Fisher Scientific	C10086	The fluorescent labeling of proliferating cells in on-plate fluorescent staining was performed along the product manual of this kit.
Annexin V, Alexa Fluor 594 conjugate	Thermo Fisher Scientific	A13203	
4',6-diamidino-2-phenylindole (DAPI)	Thermo Fisher Scientific	D1306	
Oct-3/4 Antibody (C-10)	Santa Cruz Biotechnology	sc-5279	
Donkey Anti-Mouse IgG H&L (DyLight 488)	abcam	ab96875	This is a secondary antibody used in on-plate fluorescent cell staining.
ECLIPSE Ti-E	Nikon		This is an inverted fluorescence microscope equipped with a CFI Plan Fluor 4x/0.13 N.A. objective lens (Nikon), CCD camera (ORCA-R2, Hamamatsu), mercury lamp (Intensilight, Nikon), XYZ automated stage (Ti-S-ER motorized stage with encoders, Nikon), and filter cubes for four fluorescence channels (DAPI, GFP HYQ, TRITC, Cy5; Nikon)
NIS-Elements Advanced Research	Nikon		This is a microscope imaging software used for automatic image acquisition.
CellProfiler, Version 2.1.0			This is a free open software for cell image analysis (http://cellprofiler.org/).
R			SOM analysis is performed by kohonen package of this software. This is freely available (https://www.r-project.org/).
Cluster 3.0			This is the open source clustering software (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm). Unsupervised hierarchical clustering is performed with this software.

Java TreeView			This open source software (http://jtreeview.sourceforge.net/) is used to visualize clustering data as a heatmap and a dendrogram.
H9 human embryonic stem cell	WiCell Stem Cell Bank	WA09	